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Eph RECEPTOR LIGAND ELF-2

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This invention encompasses an isolated DNA encoding all or a portion thereof of a novel member, ELF-2, of a family of ligands of the Eph receptor tyrosine kinases, along with the isolated polypeptides expressed by the DNA. The invention further encompasses DNA encoding forms of the ELF-2 ligand and polypeptides corresponding thereto. The ELF-2 ligand is important in cellular communication during pattern formation in embryos and can be used to alter neurological development, oncogenesis, and growth regulation.

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Eph Receptor Ligand ELF-2

Background of the Invention

Signal transduction by transmembrane proteins from the extracellular environment to the cell interior is critical for cell proliferation, migration, and differentiation. Ligands provide the trigger for these intercellular signals when they bind to cell surface receptors.

The tyrosine kinase receptors are a family of cellular proteins that were originally identified as the transforming gene products of some oncogenic retroviruses. The peptide growth factors that are ligands for these receptors exert powerful effects on cellular proliferation, survival, adhesion, migration, and differentiation, cell to cell signaling, axon guidance and the control of tissue organization during vertebrate development, as well as in diseases such as cancer. Cantley *et al.* (1991) *Cell* 64:281-302; Fantl *et al.* (1993) *Ann. Rev. Biochem.* 62:453-481. Following ligand binding, the intrinsic kinase activity of the tyrosine kinase receptors is typically up-regulated. Ullrich, A. and J. Schlessinger (1990) *Cell* 61:203-212.

Several of these ligands and their receptor tyrosine kinases have been the subjects of numerous functional studies and their roles are well defined. For example, it is well established that ligand KL and its receptor c-Kit have determinant roles in the proliferation, differentiation or migration of hematopoietic stem cells, neural crest progenitors, and primordial germ cells.

Until recently, however, the largest family of receptor tyrosine kinases, the Eph family, were identified as orphan receptors without known ligands. For that reason, the biological functions of the Eph ligands and

their receptors remains poorly defined.

The tissue distributions of Eph family members during embryogenesis indicate that they have of a wide variety of roles, including rhombomere segmentation and, in a smaller number of cases, somitogenesis. For example, the mouse Eph family receptors Sek, Mek4, Eck/Sek-2, Nuk/Sek-3 and Sek-4 all display rhombomere-specific expression patterns in the developing hindbrain. Becker *et al.* (1994) *Mech. Develop.* 47:3-17; Gilardi-Hebenstreit *et al.* (1992) *Oncogene* 7:2499-2506; Nieto *et al.* (1992) *Development* 116:1137-1150; Cheng, H. and J.G. Flanagan (1994) *Cell* 79:157-168. Sek1, Sek-3 and Sek-4 all display expression patterns specific for rhombomeres 3 and 5, and Eck/Sek-2 is expressed in a transverse stripe in the pre-segmented hindbrain, in a region destined to form rhombomere 4. Nuk/Sek-3 expression is restricted to rhombomeres 2, 3 and 5 as well as to specific bulges in the forebrain and midbrain.

Members of the Eph receptor family are also expressed in adult tissues and cell lines where it has been suggested they play a role in many physiological processes, including growth regulation, neurological development, differentiation and oncogenesis. Kozlosky *et al.* (1995) *Oncogene* 10:299-306; Beckmann *et al.* (1994) *EMBO J.* 13:3757-3762. However, there is little evidence that directly supports these functions.

Knowledge of specific Eph receptor ligands is essential to understanding the signaling pathways activated by ligand/receptor interactions, especially in the areas of neurological development, growth regulation and oncogenesis.

Summary of the Invention

The invention described herein relates to isolated DNA of vertebrate origin, including DNA of mammalian origin, referred to as ELF-2 genes, which encodes Eph receptor

ligands, ELF-2, as well as to functional equivalents thereof. It further relates to isolated polypeptide ligands encoded by the DNA. The term ELF-2 gene, as used herein, includes human ELF-2 genes, murine or chicken ELF-2 genes, such as the sequences (SEQ ID NO:1 and SEQ ID NO:3, respectively) represented herein, orthologous genes from other vertebrate species, and other polynucleotides which are substantially homologous to and which are the functional equivalent of the murine or chicken sequences. By functional equivalent, it is meant that the DNA encodes a product which selectively binds to an Eph receptor and demonstrates the pattern of expression and biological activity exhibited by the binding of a naturally-occurring (native) ELF-2 polypeptide to an Eph receptor in vertebrate cells or tissue.

Also provided are probes and primers, which are fragments or portions of the DNA described herein and comprise 14 or more nucleotides, as well as DNA or RNA complementary to the DNA. In addition to the isolated genomic murine DNA and the isolated chicken cDNA, included herein are the amino acid sequences of corresponding ligands and the encoded orthologous polypeptides from all vertebrate animals. Thus, this invention provides a ligand which binds selectively to an Eph receptor, especially a ligand of vertebrate origin, as well as mouse or chicken DNA that encodes the ligand corresponding to the amino acid sequences described.

Other nucleic acids of the invention include oligonucleotides related to, but distinct from, oligonucleotides of the mRNA transcribed from the ELF-2 genes and oligonucleotides complementary to such mRNA sequences, such as antisense oligonucleotides that can be used to inhibit the activity of ELF-2 in a number of ways. As described more fully below, the nucleic acids of the invention also include modifications of ELF-2 genes and

their complements.

In another aspect, the invention provides fusion proteins, especially an ELF-2-alkaline phosphatase fusion protein (ELF-2-AP), as well as methods of using such fusion proteins to detect and identify receptors, sites of ligand activity, and ligand/Eph receptor interactions.

The invention also encompasses host cells which contain the above-described nucleic acids, which are expressed in the host cells, methods of producing the encoded ligand and methods of diagnosis and treatment which make use of the DNA and encoded ligands, as well as antibodies which are specific for ELF-2 or other products and which can be used to detect, enhance or inhibit ELF-2 activity.

The DNA encoding ELF-2 ligands and the encoded polypeptides have many uses. For example, the DNA encoding all or a portion of murine or chicken ELF-2 can be used to characterize the corresponding ligand, the Eph receptor, and the cellular responses that result from the activities of the receptor when it is bound and when it is not bound to the ligand ELF-2.

ELF-2 polypeptides can be used as immunogens for the production of antibodies or antibody fragments which are specific for ELF-2. An ELF-2 polypeptide can be produced through standard transfection and/or transformation methodology and used in therapeutically effective amounts in pharmaceutical compositions, or through gene therapy, to treat mammals for conditions wherein the polypeptide or a portion thereof would improve the condition. A therapeutically effective amount of the polypeptide or a portion thereof, e.g. a secreted form of ELF-2, or a drug that mimics ELF-2 activity (an agonist) can be used in pharmaceutical compositions for treating individuals who would benefit from increased levels of ELF-2, or to stimulate the formation, and thus the activity, of the ELF-

2/Eph receptor complex. Similarly, preparations that inhibit or interfere with the activity of ELF-2 (antagonists), including antisense or dominant inhibitor-type sequences, can be used to treat individuals who would benefit from reduced levels of signal transduction mediated by ELF-2 activity.

In another aspect of this invention, an ELF-2 gene or the encoded polypeptide can be used in diagnostic assays, including immunoassays, to determine if there is an altered or mutated form of the native gene or native polypeptide, or an altered level of expression (increased or decreased) of the native gene or native polypeptide which results in or is characteristic of an abnormal condition, such as altered neurological development or tumor formation. This invention further relates to methods of detecting the level and location of expression of ELF-2 as a means of diagnosing a predisposition to the abnormal development of a mammalian embryo.

Transgenic gene therapy is also provided by this invention. In this embodiment, an ELF-2 gene or a fragment thereof is introduced in a sense or antisense orientation to affect the normal or abnormal formation or functioning of the ELF-2/Eph receptor complex in mammalian cells or tissues.

In a further aspect, this invention provides recombinant ELF-2 polypeptides and methods for producing such polypeptides in host cells. Thus, the invention provides recombinant vertebrate ELF-2 polypeptides; these recombinant ELF-2 polypeptides include recombinant ELF-2 polypeptides identical to naturally-occurring (native) ELF-2 polypeptides, and recombinant ELF-2 polypeptides which differ from naturally-occurring ELF-2 polypeptides by one or more amino acid substitutions, deletions, or insertions. The methods used for producing such recombinant ELF-2 molecules are known to those of skill in the art and

comprise transforming into a host cell (by transfection or infection) an expression vector that encodes an ELF-2 polypeptide of the invention and a suitable regulatory sequence, such as a promoter, culturing the host cells transformed with the vector under conditions such that the ELF-2 polypeptide or a modification thereof, capable of combining with its Eph receptors, is expressed.

This invention also relates to methods used to determine the receptor(s) that normally bind to ELF-2 in vertebrate cells. Both *in vitro* and *in vivo* experiments can be carried out to ascertain the effects of ELF-2/Eph receptor binding, and include the production of transgenic animals (vertebrate and invertebrate) as model systems. The understanding and characterization of the ELF-2 receptors is useful in elucidating the mechanism by which ELF-2 effects signal transduction, thereby producing intercellular communication, as well as the effect of this communication. In addition to other important findings, this will advance the knowledge of neurological development and tumor formation in vertebrates.

Brief Description of the Figures

Figure 1A-1B is the nucleotide sequence of mouse ELF-2 (SEQ ID NO:1) and the deduced encoded amino acid sequence (SEQ ID NO:2).

Figure 2A-2D is the nucleotide sequence (a) of chicken ELF-2 (SEQ ID NO:3), DNA (SEQ ID NO:4) complementary (b) to the chicken nucleotide sequence, and the deduced encoded amino acid sequence (SEQ ID NO:5).

Detailed Description of the Invention

Until recently, ligands had not been identified for the Eph receptor family, limiting knowledge of their function. The recent identification of five ligands for this family further points to important roles in

development for these kinases. The molecule B61, a cDNA originally cloned as encoding an early response factor for tumor necrosis factor-alpha, has been identified as a ligand for the Eck receptor after purification through receptor affinity chromatography. Bartley *et al.* (1994) *Nature* 368:558-560. Cheng and Flanagan (1994), *supra*, using receptor-alkaline phosphatase fusion proteins, identified ELF-1 as a ligand for the Mek4 and Sek receptor tyrosine kinases. Fusions of receptor extracellular domains to immunoglobulin Fc were used to identify ELK-L/LERK-2/Cek5-L as a ligand for the Cek5 and Elk receptors, and Ehk1-L as a ligand for the Ehk1 and Eck receptors. Davis *et al.* (1994) *Science* 266:816-819; Beckmann *et al.* (1994) *EMBO J.* 13:3757-3762; Shao *et al.* (1994) *J. Biol. Chem.* 269:2666-2669. Most recently, LERK4 has been isolated and identified as a ligand for the Hek and Elk receptors. Koslosky *et al.* (1995) *Oncogene* 10:299-305.

These five known Eph family ligands clearly form a family, displaying very significant similarity to each other. All the ligands described to date can exist in membrane-associated forms. Four of them are anchored by a glycosyl phosphatidylinositol (GPI) linkage, and one of them, Elk-L/LERK-2/Cek5-L, has a transmembrane domain.

Because eleven Eph family kinases are now known, it is likely that many ligands for this important family currently remain unidentified. Applicants provide herewith the genes encoding a new family member ligand, ELF-2, and the encoded polypeptides. ELF-2 is of vertebrate, including mammalian origin, and encodes a polypeptide which binds the Eph receptor (*i.e.*, encodes a polypeptide which is an Eph receptor ligand).

Examples of ELF-2 genes and polypeptides include DNA which has been isolated from a library of newborn mouse brain cDNA and the encoded protein which is expressed in the specific areas of the developing embryo. Further

provided is the gene sequence of ELF-2 cDNA from a chicken library and its encoded polypeptide.

The amino acid sequence of mouse ELF-2 encodes a transmembrane molecule, more similar to LERK-2 than to the other four known Eph family ligands. *In situ* hybridization analysis of ELF-2 shows strong expression of ELF-2 in the hindbrain region and in organizing somites of 8.5 to 10.5 day murine embryos, which means that it is temporally and spatially positioned to interact with several Eph family receptors.

Cloning and Sequencing of ELF-2

Applicants have described the sequence of ELF-1, a ligand for the Mek4 and Sek receptor tyrosine kinases. Cheng and Flanagan (1994) *supra*. A search of the DBEST expressed sequence tag database for nucleic acid sequences homologous to ELF-1 revealed a 337 bp human cDNA fragment which shares 41% identity with ELF-1, over a stretch of 39 amino residues. To investigate further whether this sequence might represent a new member of the Eph ligand family DNA having the sequence was isolated by polymerase chain reaction (PCR) from a human brain cDNA library. The human cDNA PCR product was then used to screen a cDNA library from mRNA of newborn mouse brain. Seven hybridizing clones were identified. Nucleotide sequencing showed that one of these clones encoded a previously known ligand, ELK-1/LERK-2/Cek5-L. The remaining six clones contained overlapping sequences that represented a novel cDNA. This cDNA comprises an open reading frame encoding a 334 amino acid polypeptide, which Applicants herein refer to as ELF-2 (Figure 1A-1B, SEQ ID NO:1).

The DNA sequence starts with a methionine codon (Figure 1A-1B, nucleotides 90-92) in a nucleotide sequence context consistent with a translation initiation site (Kozak, M. (1987) *Nucleic Acids Res.* 15:8125-8148),

followed by a potential signal peptide for secretion (von Heijne, G. (1990) *J. Membrane Biol.* 115:195-201). The region between amino acid residues 226 and 251 is hydrophobic and likely represents a transmembrane domain.

The 224 amino acid-residue extracellular domain displays close homology to other members of the ligand family, particularly ELK-L/LERK-2/CEK5-L, and includes the four cysteine residues that are conserved in all known members of the Eph ligand family. However, as the murine ortholog of ELK-L/LERK-2/CEK5-L has already been isolated (Shao *et al.* (1994) *supra*), it is clear that ELF-2 represents a novel ligand. An alignment with ELF-2 gives an overall homology of 60% amino acid identity for the mature peptides, excluding the secretion signal sequences. A core sequence (residues 31 to 155) shared amongst all the members of the family, that includes four conserved cysteine residues and is likely to be involved in receptor binding, shows 61% identity between ELF-2 and ELK-L/LERK-2/CEK-L. A particularly remarkable aspect of the homology of ELF-2 with ELK-L/LERK-2/CEK5-L is the close homology of their intracellular domains. In a comparison of the two sequences, the 83 amino acid residue intracellular domain displays an overall amino acid identity of 75% with the last 33 amino acid residues being completely identical.

In one of the six ELF-2 clones isolated, E2.1, 93 bp are deleted. This small deletion does not disrupt the reading frame. This region corresponds exactly to the third exon of the LERK-2 gene (Fletcher *et al.* (1994) *Oncogene* 9:3241-3247), suggesting that it represents an alternate splice form. The splice form would produce a peptide lacking the amino acid residues from residue 137 to residue 168, which includes one of the four cysteine residues that are highly conserved throughout this family of ligands.

To isolate the chicken homologue, a probe comprising a

portion of the mouse ELF-2 DNA sequence was used to screen a cDNA library from chicken. The chicken ELF-2 cDNA shown in Figure 2A-2D comprises a nucleotide sequence (a) of 1712 nucleotides (SEQ ID NO:3) which encodes an ELF-2 polypeptide (SEQ ID NO:5).

Hybridization Analysis of the Temporal and Spatial Expression Patterns of ELF-2

Ligands to the Eph family receptors are of particular interest because the receptors display highly characteristic expression patterns during early vertebrate development. Eph receptors are also expressed in most adult tissues and cell lines, indicating that ligands also play important roles in the physiology and maintenance of the adult body.

Because of the unique functions suggested by these expression patterns, Applicants tested the temporal and spatial expression patterns of murine ELF-2 in the embryo. *In situ* hybridization analysis of whole mount mouse embryos was performed at days 8.5, 9.5 and 10.5 of development. As hybridization was performed using digoxigenin-labeled probes, regions of ELF-2 expression were indicated by purple staining of the whole mounts. Analysis was performed using three different probes, one corresponding to bases 348 to 981 in the ELF-2 protein-coding sequence and the other two corresponding to the 3' untranslated region. All three probes gave the same expression pattern. For comparison, *in situ* analysis was also performed for the Sek receptor tyrosine kinase.

ELF-2 expression in embryos at 8.5 days of development, soon after the onset of organogenesis, is concentrated in the hindbrain, the branchial arches and as two intense bands in the region of somite formation at the boundary between the pre-somitic and somitic mesoderm. Weaker staining is apparent in the segmented mesoderm and

within restricted regions of the forebrain. The staining in the region of the newly formed somites is strikingly similar to that of Sek. Sek and also Sek-4 were previously reported to be expressed in a wave down the embryo associated with each newly forming somite. Nieto *et al.* (1992) *Development* 116:1137-1150; Becker *et al.* (1994) *Mechan. Dev.* 47:3-17. In particular, Sek expression is seen prominently in two stripes: one in the somite currently condensing, and another in the anterior portion of the most recently formed somite. ELF-2 expression is similarly seen most prominently in a pair of bands. The posterior band is wider, and is in or near the somite undergoing condensation, while the more anterior band is narrower, although often more intense, and is in or near the most recently formed somite. However, Sek and ELF-2 differ in the lack of expression of ELF-2 in the prosomitic mesoderm.

ELF-2 expression in the hindbrain is strongest in the anterior region. Like several of the Eph family receptors, ELF-2 shows a segment-specific pattern of staining in the hindbrain. The expression of ELF-2 appeared strongest at the level of rhombomeres 1, 2, 4, and 6, and weakest in rhombomeres 3 and 5. When viewed dorsally, it is apparent that ELF-2 expression is mostly confined to the dorsal/lateral region of the open neural folds, and is absent from the midline.

In embryos at 9.5 days of development, ELF-2 expression continues in all the regions noted for 8.5 day embryos. Although less obviously segmented, the staining of the hindbrain remains strongest anteriorly. The two bands of expression near the border between somitic and pre-somitic mesoderm remain clearly visible, with the anterior band generally narrower and often more intense. Strong expression remains in the branchial arch, while weaker expression is seen in the segmented mesoderm and in

the forebrain, and is now also apparent in the midbrain, particularly toward its anterior end.

In embryos at 10.5 days of development, ELF-2 is most strongly expressed as bands in the region of somitogenesis. While many 10.5 day embryos display the same pattern in this region as described for the earlier embryos, in a large proportion of embryos the posterior band is considerably fainter than the anterior band, and additional bands can be seen in more anterior somites. Staining of the hindbrain is reduced by day 10.5, compared to earlier stages. Expression is also apparent in the branchial arches, the forebrain and near the optic cup, and also in the limb bud, in a band adjacent to the prominent distal band of Sek expression.

Binding of ELF-2 Fusion Protein to Eph Receptors

To test for ligand-receptor binding interactions, Applicants constructed a fusion protein in which part of the murine ELF-2 polypeptide was used to produce a soluble, tagged affinity reagent that could be used to detect ligands, receptors, or ligand-receptor interactions. See Flanagan, J.G. and P. Leder (1990) *Cell* 63:185-194. The ELF-2 ligand extracellular domain was genetically fused to human placental alkaline phosphatase (AP). The AP provided a tag that binds to antibodies and, more significantly, had an intrinsic enzyme activity that could be used to trace the molecule without purification, chemical labeling, or the use of secondary reagents such as antibodies. The ELF-2 fusion protein (ELF-2-AP) was then used as an affinity reagent to determine its binding characteristics to various Eph receptors.

The ELF-2 fusion protein was tested for binding to cells transfected with plasmids encoding one of six different Eph receptors: Cek10, Cek5, Elk, Cek9, Mek4 and Sek (See Example 4). The first three receptors (Cek10,

Cek5 and Elk), showed obvious binding to the fusion protein. Of these three, the affinity for Cek10 seems to be highest; however, the dissociation constants for all receptors are in the nanomolar range, consistent with functionally significant ligand-receptor interactions. The last three receptors (Cek9, Mek4 and Sek) did not show obvious high-affinity binding, although it is possible that there was some interaction.

The binding data, combined with the known expression data, suggests there may be interaction of ELF-2 with Cek10/Sek4/Hek4 in the hindbrain and the developing somites, as well as interaction with Cek5/Sek3/Nuk in the hindbrain during development. Elk receptor expression in the embryo has not been examined in detail, therefore it is difficult to determine if there is an ELF-2/Elk interaction in the developing embryo.

Expression of Cek10, Cek5, and Elk has also been detected in adult brain and several other tissues (Tuzi, N.L. and W.J. Gullick (1994) *Br. J. Cancer* 69:417-421), suggesting that ELF-2 could modulate the activity of cells in adult animals.

Functional Analysis of ELF-2

Applicants have isolated vertebrate cDNA encoding a novel member of a family of ligands of the Eph-related tyrosine kinases. The molecule encoded by the murine cDNA, designated an ELF-2, shows homology to all the known members of the Eph ligand family. ELF-2 is most closely related in primary sequence to ELF-L/LERK-2/CEK5-L, and these two are also the only two known members of the family with a transmembrane domain. Both can bind to the Elk receptor.

The close homology of ELF-2 and ELK-L/LERK-2/CEK5-L is evident in the extracellular domain, likely to bind receptor, and also in the intracellular domain, where the

two proteins show a complete identity of the last 33 amino acid residues. This remarkable level of conservation between the two sequences suggests an important role for the carboxy termini of both proteins. One possible function of the intracellular domain could be a role in regulating the ligand-receptor interaction. In this context, it is interesting that receptor activation mediated by ELK-L/LERK-2/CEK5-L and EHK1-L apparently requires that the ligands be either clustered or presented on cell surfaces. Davis *et al.* (1994) *Science* 266:816-819. Therefore, the ligand intracellular domain could function to facilitate or regulate ligand oligomerization. An alternative function for the intracellular domains of ELF-2 and ELK-L/LERK-2/CEK5-L could be associations with other proteins that can mediate the transmission of a signal into the interior of the ligand-presenting cell.

The temporal and spatial study of the expression of Eph ligands and Eph family receptor tyrosine kinases provides important clues to the function of these molecules in development. While developmental expression studies have been reported for many of the kinases, limited information is so far available for the ligands, and *in situ* hybridization analysis has so far been reported only for ELF-1. Cheng and Flanagan (1994) *supra*.

The *in situ* analysis provided here for ELF-2 suggests several possible functions. The expression of ELF-2 during the day 8.5 to day 10.5 period of development, temporally positions ELF-2 to play roles in early organogenesis. Clearly, the expression of ELF-2 in the hindbrain, and more weakly in the midbrain and forebrain, indicates a role for ELF-2 in the development of the nervous system. Many Eph family kinases display rhombomere-specific expression in the hindbrain, which has implied a role in hindbrain segmentation. The expression of ELF-2 in this region suggests it may interact with one or more of these kinases

and play a role in either the establishment of the segmental pattern or in the subsequent development of segment specific properties such as neural crest emigration or axon guidance. In view of the binding data, receptors for an interaction with ELF-2 in the hindbrain would include Cek5/Sek3/Nuk and Cek10/Sek4/Hek4.

The expression of ELF-2 at the site of somitogenesis indicates its role in mesoderm segmentation. Similar bands appear in Sek and Sek-4 expression which initially occurs in the condensing somite, and is down-regulated as the formation of the somite is completed. The expression of ELF-2 in similar regions therefore indicates the involvement of ELF-2 in somite formation. Based on the binding data and expression patterns, the interaction of ELF-2 with Cek10/Sek4/Hek4 receptors is possible in this region.

Characterization and Uses of ELF-2

This invention relates to DNA encoding ELF-2 ligands of vertebrate origin, to the encoded ELF-2 proteins, to the functional equivalent of the DNA and/or proteins, and to the use of all of these molecules in diagnostic, assay, screening, and therapeutic procedures, in methods to identify other ligands that bind to Eph receptors, and in methods of promoting, inhibiting, and otherwise altering neurological development and regulating growth in vertebrate animals.

The functional equivalent of ELF-2 ligands of other vertebrates comprises a polypeptide which: (1) has greater than 70% sequence homology to the mouse or chicken ELF-2 ligand; (2) binds the native ELF-2 Eph receptors for that species; and (3) demonstrates similar embryological temporal and spatial expression patterns on hybridization analysis as those observed and described for ELF-2 in the mouse embryo.

Practice of this invention can employ DNA which comprises the nucleotide sequences depicted in Figure 1A-1B (SEQ ID NO:1), Figure 2A-2D (SEQ ID NO:3 AND SEQ ID NO:4), or an ELF-2 gene which encodes a mature ELF-2 protein. This includes orthologs of the murine or chicken ELF-2 genes and their encoded protein ligands, and other functionally equivalent DNA (comprising 100 or more nucleotide residues) and polypeptides produced as a result of mutagenesis, substitutions, and other molecular biology techniques known to those skilled in the art. DNA molecules which differ in codon sequence from the DNA sequences described above due to degeneracy of the genetic code are also included. The terms "ortholog" or "orthologous" as used herein refer to genes and proteins which are homologs by speciation; that is, they have a common descent based on structural and functional characteristics. Fitch, W.M. (1970) *Syst. Zool.* 19:99-113.

This invention also encompasses DNA and/or RNA which hybridizes to the aforesaid DNA, under moderate conditions and particularly under stringent conditions (see, Ausubel *et al.* (1994) *Current Protocols in Molecular Biology*, Section 6.4, John Wiley & Sons, NY), and which encodes, on expression, an ELF-2 ligand protein, or a portion thereof, in a vertebrate cell. A hybridization analysis, as described in Ausubel *et al.* (1994) *supra*, can be used to detect complementary polynucleotides.

This invention further encompasses cDNA sequences encoding vertebrate ELF-2 protein, and methods of making and using the ELF-2 protein, including, but not limited to, vertebrate model systems such as transgenic and knockout mice, as well as for genetic mapping studies.

Thus, this invention provides for the first time isolated, purified ELF-2 ligand protein, free or substantially free from other proteins with which it is associated in nature. The ELF-2 protein may be used as an

affinity reagent for the identification or purification of its Eph receptor, or for the production of polyclonal or monoclonal antibodies which may, in turn, be used therapeutically as well as in diagnostic and assay procedures. Alternatively, the sequence can be used to design probes to isolate homologous protein ligands from other species.

The invention provides fusion proteins which are chimeric proteins produced by a fusion of cDNA encoding an ELF-2 polypeptide or a portion thereof with cDNA encoding another amino acid sequence that is not related to or homologous to the ELF-2 sequence. The resulting fusion proteins encoded by this construct can be used essentially like antibodies to detect ligands, receptors, or ligand-receptor interactions. There are certain major advantages to using these fusion proteins, for example: (1) they can be used to search for previously unknown receptors; (2) they can detect all sites of receptor activity (whereas antibodies only detect the particular molecule they were raised against); and (3) since they detect natural ligand-receptor interactions, they can be used in a screen to test for agonists or antagonists of the interaction. Further, the alkaline phosphatase tag can be bound to available monoclonal antibodies and also provides the fusion protein with an intrinsic enzyme activity than can be used to trace the protein with high sensitivity by quantitative or *in situ* assays. Examples of receptor-AP fusion proteins can be found in Cheng and Flanagan (1994) *supra* (Mek4-AP and Sek-AP), and Flanagan and Leder (1990) *supra* (APtag-Kit).

The ELF-2-AP fusion protein, described above and in Example 3, is a particularly useful example of this technology. It can be used to identify Eph receptors to which ELF-2 can bind (see Example 4), and to determine their cellular location, thereby indicating the pattern of ELF-2 expression in an organism. Therapeutically, once

normal expression patterns of an organism have been determined, it can be applied to detect and assay abnormal expression or to monitor the effects of treatment involving modifications of ELF-2 or receptor activity. Either *in vitro* or *in vivo*, it can be used to study the effects of mutations and alterations of ELF-2 or its receptors, as well as the effects of potential agonists and antagonists on ELF-2 or its receptors. This protein has already provided valuable information through the results of the binding experiments which demonstrate that the transmembrane and intracellular domains of ELF-2 are not essential for receptor binding.

In the same manner (see below) that antibodies and probes can be used for quantitative assays, for diagnostic procedures, and to monitor the treatment of individual conditions, fusion proteins can be substituted as a highly sensitive and easily monitored tagged reagent for the same purposes. Furthermore, diagnostic kits using fusion proteins can be developed for detecting and quantifying ELF-2 activity in cells and tissues of mammals.

ELF-2 expression in cell or tissue samples can be detected using DNA or RNA probes which would be indicative of signal transduction or other activities resulting from formation of an ELF-2/Eph complex. For example, a sample of brain tissue from an individual can be examined for hybridization to a DNA probe which is complementary to all or a portion of an ELF-2 gene. Detection of hybridization is an indication of signal transduction which, in turn, indicates the occurrence of developmental processes such as axon guidance or neural development. The probe can consist of an isolated oligonucleotide comprising 14 or more consecutive nucleotide residues in a sequence that hybridizes under stringent conditions to the DNA shown in Figure 1A-1B (SEQ ID NO:1), Figure 2A-2D (SEQ ID NO:3 and SEQ ID NO:4), or to the sequence of another ELF-2 gene or

its complement. Ausubel *et al.* (1994) *supra*.

Probes or primers can also be labeled with radioisotopes, enzymes, enzyme co-factors, fluorescent compounds, and the like by methods known to those of skill in the art. Sambrook, *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Labeled probes can be used diagnostically to ascertain the presence of ELF-2 genes in a cell or tissue, or for detecting the level of mRNA encoding ELF-2 in cells or tissues to determine whether an ELF-2 gene is functioning normally or abnormally or not at all.

Alternatively, expression of the ELF-2 protein can be detected using polyclonal antibodies, monoclonal antibodies, or antibody fragments such as $F(ab)_2$ fragments. Given an ELF-2 gene, ELF-2 polypeptide, or a functional equivalent, antibodies or antibody fragments can be produced by standard methods. Harlow, E. and D. Lane (1988) *Antibodies - A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor Laboratories, NY. The antibodies of the invention include bifunctional, chimeric or humanized antibodies, antibody fragments, and can be used in immunoassays and diagnostically, as well as for treatment purposes. For example, the cerebral spinal fluid or brain tissue sample from an individual can be contacted with antibodies specific for human ELF-2. Detection of the antibody/antigen complex in cells is an indication of ELF-2 and therefore, activity due to intercellular signals. Such immunoassays can also be used to quantitate antigens and antibodies where extreme sensitivity is required, and to monitor the progress of treatment in procedures employing enhancers or inhibitors of ELF-2.

In many instances, the antibody can be labeled or a second antibody that binds to the first antibody can be

labeled by some physical or chemical means. The label may be an enzyme which is assayed by the addition of a substrate which upon reaction releases an ultraviolet or visible light-absorbing product or it can be a radioactive substance, a chromophore, or a fluorochrome. E. Harlow and D. Lane (1988) *supra*.

Antibodies can also be constructed to inhibit the binding of ELF-2 to its Eph receptor. For this purpose, therapeutic antibodies such as mouse/human chimeric monoclonal antibodies, humanized antibodies, bifunctional antibodies, or antibody fragments such as $F(ab)_2$ fragments are particularly useful, since they can be specifically designed to bind to the extracellular binding domain of the ligand and may be utilized for *in vivo* human therapy. Further determining which antibody sequences prevent binding by the receptor will help to ascertain how receptor binding affects the cellular communication functions of the receptor/ligand complex.

The present work also provides a method of determining the location and the activity of the ELF-2/Eph receptor complex. When activated by mutation or overexpression, both ligands and receptor tyrosine kinases can become potent oncogenes and cause drastic cellular transformation. Cantley *et al.* (1991) *Cell* 64:281-302. The above-described methods can be used diagnostically to determine if ELF-2/Eph receptor activity has been altered from normal levels in cells and tissues, thus providing a powerful tool for the detection of tumors.

For example, a cell or tissue sample can be obtained from an individual to be assessed for the likelihood of tumor formation, e.g., cancer. The sample can be treated in such a manner that DNA in cells in the sample is rendered available for hybridization with complementary DNA, such as an ELF-2 probe, or proteins in the cell are

available to be bound by an antibody, and the resulting hybridization complex or antibody-antigen product detected and measured. A known sample of normal (known to be unaffected or unaltered) cells or tissue may be used as a control. The amount of ELF-2 measured compared to the control cell or tissue sample is an indication of the alteration in ELF-2/Eph receptor activity and thus, tumor development. Antibodies or fusion proteins, labeled as described above, may be especially useful for this purpose.

Probes and primers may also be used to diagnose and detect the presence or absence or alteration in ELF-2 activity as an indication of disease or abnormal development, especially in a developing fetus. Such methods may be used to monitor the progress of development as well.

In a further aspect, this invention provides a diagnostic kit for carrying out a method of detecting an ELF-2 ligand and level of ELF-2 activity in cells and tissues of mammals. The method of detection involves hybridizing a sample of genomic mRNA encoding an ELF-2 ligand from an individual with one or more hybridization probes, wherein the kit includes a hybridization probe and one or more other components for carrying out the method, characterized in that the hybridization probe is as defined above in that it is able to bind to mRNA encoding the ELF-2 ligand in a cell. The probe may be labeled for easier detection and for quantification purposes.

In many embodiments, ELF-2 encoding DNA or another nucleotide sequence of this invention is inserted into a plasmid or other vector construct and linked to vector DNA and various genetic elements advantageous for selection, transcription control, amplification, and other procedures. The ELF-2 encoding DNA is preferably expressed in vertebrate host cells, although expression in bacterial, yeast and cells of invertebrates may be effected using

standard methods and procedures (Sambrook *et al.* (1989) *supra*).

Eukaryotic expression vectors into which the DNA of the invention is inserted, with or without additional linkage components, can be synthesized by techniques well known to skilled artisans. Sambrook *et al.* (1989) *supra*. These expression vectors may also utilize inducible promoters or comprise inducible expression systems such as those described in the art. See, e.g., International Application No. PCT/US87/01871.

Established cell lines, preferably vertebrate cell lines, are suitable as hosts. These can be 3T3 cell lines derived from Swiss, Balb-c, or NIH mice for transient expression, or COS-1 monkey cells for stable expression of proteins in eukaryotic cells. Other useful cell lines include CHO (Chinese Hamster Ovary) cells, C127 mouse cells, HeLa cells, melanoma cell lines, mouse L929 cells, BHK or HaK hamster cell lines, and the like.

Plasmid DNA can be prepared by conventional methods and ligated to the DNA sequences of this invention. Examples of plasmids useful for this purpose are pcDNAI (Invitrogen, San Diego CA) for transient expression and LTR Moloney murine leukemia virus for stable expression of proteins. Transfected cells, preferably vertebrate, that are capable of expressing the cDNAs of this invention can provide cell lines for production of the ELF-2 protein and portions thereof, or as part of a gene therapy protocol to deliver any of the nucleotides of this invention. Appropriate transfection or transformation, selection, amplification, cell culture and recovery methods are conventional and well known to skilled artisans in this field. Both viral and non-viral vector systems can be used to deliver and express genes in specific vertebrate tissues. See, for example, Ausubel, *et al.* (1993) Section 9, *supra*. and other molecular biology laboratory manuals.

The expression domains of the receptor tyrosine kinases include the vertebrate development stages of gastrulation and early organogenesis. However, little is known about signal transduction between cells carried out by these receptors and their ligands although these activities may mediate some of the earliest patterning events in development. Further, the Eph receptors have been implicated in the development and maintenance of nearly every tissue in the vertebrate body, as well as having a role in tumorigenesis.

The nucleic acids and polypeptides of this invention provide the means by which the activity of the ELF-2/receptor complex can be studied and manipulated *in vitro* or in embryo systems. For example, coding sequences for an ELF-2 protein or a portion can be incorporated with different promoters into vectors and then delivered into various cell systems to determine the effects of differential expression. Or an ELF-2 protein can be modified through mutation, or deletion, addition, substitution or other modification of specific nucleotide residues or coding sequences of the ELF-2 gene. The resulting gene product can then be incorporated with appropriate regulatory sequences into cell systems for study of the functional effects of these changes to the polypeptide.

Pharmaceutical agents which interfere with the formation or stability of ELF-2 signaling mechanisms (such as those comprising an ELF-2/Eph receptor complex), can be used to treat or prevent diseases or their pathological effects mediated by such complexes. Until now, nothing was known of ELF-2 protein or its role in neurological development. Applicants have shown that, in the mouse, this is a 334 amino acid transmembrane protein that is highly expressed in the hindbrain region and in segmenting mesoderm in mouse embryos indicating an important role in

the development of the nervous system. This knowledge allows procedures such as competitive binding assays to be used to determine the effects of potential agents on the binding of ELF-2 to its receptor tyrosine kinase. In fact, in one embodiment of this invention, mouse, chicken or other vertebrate embryos can be used to test the effects of such agents on neurological development and on somite formation, and wherever else ELF-2 may be highly expressed during embryological development. Those binding agents which bind to ELF-2 Eph receptors but which do not bind (or bind with low affinity) to Eph receptors that do not bind to ELF-2 are of particular interest. They will provide a basis for pharmaceutical agents useful as highly specific antagonists of ELF-2 activity.

Therapeutically, ELF-2 could be used to alter cellular activities in adult animals even in cells that do not normally have ELF-2 activity. The expression of the three receptors that bind ELF-2 (Cek10, Cek5 and Elk) has been detected in adult tissues as well as embryos, suggesting that ELF-2 can modulate the activity of cells in adult animals whether or not it is normally expressed in these tissues.

Other compounds may be used to specifically inhibit or interfere with the activity of ELF-2 or an ELF-2/Eph receptor complex. Antisense oligonucleotides, for example, can be used to inhibit the expression of ELF-2 and thus prevent signal transduction. These oligonucleotides can be DNA or RNA. The DNA oligonucleotides can be operably linked to a constitutive, tissue-specific, or environmentally-triggered regulatory sequence so that transcription occurs at a specified time and/or cellular site. Such a construct can be particularly useful to prevent or inhibit the growth of tumors that may result from ELF-2/receptor activity. Alternatively, mRNA oligonucleotides can be introduced directly, either

systemically or into cells or tissues by known methods. Dominant inhibitor methodology can be used for the same purposes. By the introduction of sense strands, the expression of ELF-2 can be inhibited.

There are also situations in which one may want to induce or enhance activity or provide an agonist of ELF-2 in cells. This procedure can be used to induce neuronal cell differentiation or to maintain the integrity of cells of the nervous system at a particular stage of differentiation. These cells can be used therapeutically for repair and regeneration of neurons in both juvenile and adult individuals.

For example, cells from fetal or neonatal animals can be transplanted into adult brains to repair damaged brain tissue. In addition to maintaining cell cultures at predifferentiation levels and helping to prevent loss of differentiation, ELF-2 or an agonist may be useful to induce differentiation of cells where they are grafted into the brain. In fact, treatment with ELF-2 by itself may induce repair of nervous tissue throughout the central and peripheral nervous systems.

ELF-2 activity could be instrumental in maintenance, performance, and aging of normal cells. Further, modulation of ELF-2 activity could be useful to prevent or treat tumor formation. If so, it is likely that pharmaceutical compositions containing ELF-2 or an agonist of ELF-2 will be useful in maintenance, repair and regeneration processes, and in the prevention of degeneration and death of such cells under certain pathological conditions. Thus, this invention also provides ELF-2 ligands or agonists by themselves or in pharmaceutical compositions for the treatment of, or prevention of, neurological pathologies. These pathologies include injuries to the nervous system such as traumatic injury, infectious and tumor-induced injury, chemical

injury, vasal injury, deficits, and the like. They also include lesions and other effects due to aging (such as Alzheimer's disease), chronic neurodegenerative diseases such as Parkinson's disease, Huntington's chorea, etc., chronic immunological diseases including multiple sclerosis, and disorders of sensory neurons. Treatment of individuals suffering from these disorders can benefit from enhancement of ELF-2 activity that mediates homeostasis of the nervous system, activates repair functions, and prevents neuronal degeneration.

Other examples wherein individuals can derive benefits of modulating ELF-2 activity involve the maintenance and generation of non-nervous cells and tissues. ELF-2 ligands, like other Eph ligands, act as induction signals for development and differentiation processes throughout the animal body. Thus, the potential exists to selectively control, maintain, and regenerate tissues from other organ systems, such as liver, lungs, muscle, heart, testes, ovaries, and the like. ELF-2 polypeptides, agonists and/or antagonists of ELF-2 can be used to halt the degeneration of cells and tissues of the lungs and liver, and to promote the repair of the same. Further, ELF-2 polypeptides can be applied to induce regeneration of damaged tissues and organs. In conjunction with transplantation methodologies, ELF-2 polypeptides or agonists can be used to promote growth and integration of both native and transplanted tissues and/or organs. Antagonists of ELF-2 polypeptides can be used at the same time to inhibit rejection of transplanted materials by immune system activities.

ELF-2 polypeptides or their functional equivalents, enhancers or inhibitors of ELF-2 polypeptides, and agonists or antagonists of ELF-2 are among the compounds included in this invention that will be useful to treat conditions arising from or affected by ELF-2 activity. These

compounds provide the basis for producing highly specific pharmaceuticals useful in controlling the adverse affects of ELF-2 ligands or in specifically enhancing ELF-2 ligand activity where it is needed.

Compounds that control or overcome or enhance the effects of ELF-2 ligands can be formulated into pharmaceutical compositions containing a pharmaceutically acceptable carrier and/or other excipients using conventional materials and means. This includes expression vectors containing nucleotide sequences that encode ELF-2 polypeptides or antisense oligonucleotides. These compositions can be administered to an animal, either human or nonhuman, for therapy of a disease or condition resulting from ELF-2 ligand activity or requiring ELF-2 ligand activity. Administration may be by any conventional route (parenteral, oral, inhalation, transplantation, and the like) using appropriate formulations, many of which are well known. The compounds can be employed in admixture with conventional excipients, such as, pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral administration that do not deleteriously react with the active derivatives. These include, but are not limited to, water, salt solution, alcohols, vegetable oils, polyethylene glycols, gelatine, carbohydrates, fatty acid esters, talc, and the like. For parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories.

It will be appreciated that the actual preferred amounts of active compound in a specific case will vary according to the specific compound being utilized, the particular compositions formulated, the mode of application, the particular situs of application, and the characteristics (e.g., age, sex, body size, weight, and the

type and severity of the condition being treated) of the individual being treated. Dosages for a given recipient will be determined on the basis of these individual characteristics as well. A therapeutically effective dose is one that will result in reduction of part or all of the adverse symptoms of the disorder.

The compounds of this invention also provide the means by which the Eph receptor for ELF-2, and homologous ELF-2 ligands and their respective Eph receptors from other species, can be cloned and isolated. Access to these compounds, which have not been previously available, provides benefits of several animal model systems (such as chickens, rats, mice, monkeys, apes, dogs, cats, horses) for the study of the role of this ligand and its receptor in many cellular processes. Also, recombinant human ELF-2 can provide antibodies for therapeutic purposes that will invoke fewer immune systems responses and, as a result, fewer side effects of such therapies.

The ELF-2 gene can be used to prepare transgenic animals for screening and testing of pharmaceuticals that affect ELF-2 activity. For example, the natural ELF-2 gene can be knocked out or replaced with a recombinant inducible gene using a plasmid inserted in a mouse embryonic stem cell. From this a transgenic mouse can be generated that can be useful as a model or test system for the study of neural development, cancer, spacial patterning in embryos, axon guidance, neural diseases, and the like. The generation of knockout mice and mouse cells makes it possible to determine the effects of inhibitors and enhancers of ELF-2 activity directly. In addition, the toxicity and the effects of an inhibitory substance on other ligand/receptor complexes can be assessed in an animal model where the specific target has been removed. Therefore, any side effects, including effects on other regulatory molecules, can be distinguished from the effects

of diminishing or deleting ELF-2 activity.

The same or another vertebrate system can be used to test the effects of therapeutic and pharmaceutical compounds on ELF-2 and ELF-2/Eph receptor complex activity. Inhibitory compounds, including antibodies, directed towards such activity in tumors and the brain, etc. can be screened for efficacies and side effects.

The following examples describe specific aspects of the invention to illustrate the invention and provide a description of the methods used to isolate and identify ELF-2 genes and ligands for those of skill in the art. The examples should not be construed as limiting the invention in any way.

Example 1

PCR Amplification and Library Screening:

The DBEST database was screened for sequences homologous to ELF-1 using the BLAST program (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410), resulting in identification of a fragment of cDNA sequence with homology to a short stretch of ELF-1. This sequence, accession number L13819, had been obtained from a human 3 month postnatal whole brain cDNA library. To perform further studies, Applicants amplified this sequence from reverse transcribed human brain total RNA (Clontech) by PCR, using two oligonucleotides:

AB180, GGAAGCTTATCAAATTCACCATCAAGTTTCAAG (SEQ ID NO:3); and

AB181, AATGTCCGGCGCTGTTGCCGTCTGTGCTAGAAC (SEQ ID NO:4).

Reverse transcription was performed using the Statascript RT-PCR kit (Stratagene). PCR Amplification was for 35 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 74°C, using Taq I DNA polymerase (Perkin Elmer Cetus). The

resulting 334 bp PCR product was purified by agarose gel electrophoresis, and was then radioactively labeled and used to screen a murine brain cDNA library by hybridization, with a low stringency wash at 50°C in 2x SSC. Seven hybridizing clones were isolated. One of them represents mouse ELK-L/LERK-2/CEK5-L. Five of them (clones E2.2 to E2.6) appeared by restriction analysis and sequencing to be overlapping clones of a single cDNA sequence, which is shown in Figure 1A-1B. The remaining clone, E2.1, contains an open reading encoding the complete ELF-2 amino acid sequence, but is missing nucleotide residues 413-505.

Example 2

In Situ Analysis of ELF-2 Expression:

Analysis of ELF-2 RNA expression was performed using three different probes, one from the protein-coding region and two from the 3' untranslated region. All three probes gave the same expression pattern. A subclone of E2.2, pELF2E, was generated containing the coding sequence from the 5' end of clone E2.2 at residue 262 to the *Pst*I site at residue 895. Two more subclones, pELF2H and pELF2I, were generated from clones E2.1 and E2.3, respectively, and both contain fragments of the 3' untranslated regions of the ELF-2 cDNA. The nucleotide sequence of this part of the cDNA has not been determined, but restriction mapping indicates this region is shared by several independent ELF-2 clones. All three subclones were used as templates for T3 and T7 RNA polymerases, to produce sense and antisense probes to ELF-2. To produce the antisense strand, pELF2E was digested with *Hind*III (in the polylinker of the pBluescriptSK used to generate the subclones) and transcribed with T3 RNA polymerase to generate a 633 bp probe. pELF2H and pELF2I were digested with *Bam*HI (also in

the polylinker) and transcribed with t& RNA polymerase to give 700 bp and 550 bp antisense probes, respectively. Polymerization was conducted in the presence of digoxigenin-UTP, to produce digoxigenin-labeled probes as described previously (Wilkinson, D.G. (1992) *supra*). Antisense probes for Sek were produced as described previously (Cheng and Flanagan (1994) *supra*). *In situ* hybridization of whole mount embryos was conducted as described by Wilkinson (1992) *supra*, with modifications as in Cheng and Flanagan (1994) *supra*.

Example 3

Construction of ELF-2 Fusion Proteins:

An ELF-2-AP fusion protein was constructed essentially as described in Cheng and Flanagan (1994) *supra* for producing Mek4-AP and Sek-AP fusion proteins using methodology described in Flanagan and Leder (1990) *supra*. The extracellular domain of ELF-2, ending at residue Glu-225, was linked to the N-terminus of human placental alkaline phosphatase (AP). The enzymatic activity of the AP tag was then available to trace the soluble fusion protein in quantitative or *in situ* assays.

Example 4

Binding of ELF-2 Fusion Protein to Receptors:

The ELF-2 fusion protein was tested for binding to cells transfected with plasmids that encode Eph family receptors according to Cheng and Flanagan (1994) *supra*. The following receptors were tested:

(1) Cek10 (a chicken sequence with orthologs Sek4 and Hek2);

(2) Cek5 (a chicken sequence with orthologs Sek3 and Nuk);

(3) Elk (a mouse sequence with ortholog Cek6);

(4) Cek9 (a chicken sequence);

(5) Mek4 (a mouse sequence with orthologs Cek4 and Hek); and

(6) Sek (a mouse sequence with ortholog Cek8).

With each of the first four Eph receptors, the extracellular domain was fused to a trk receptor intracellular domain. However, this would not affect the basic results.

Example 5

Isolation of ELF-2 ligand cDNA from Chicken

Radioactive probes prepared from the mouse ELF-2 DNA sequence were used to screen a chicken cDNA library. The chicken ELF-2 DNA was identified and isolated in accordance with procedures described in Sambrook, *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

All publications and references cited are herein incorporated by reference.

Equivalents:

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

Claims

We claim:

1. An isolated polypeptide encoded by DNA selected from the group consisting of:
   (a) DNA comprising SEQ ID NO:1 or SEQ ID NO:3;
   (b) a nucleotide sequence which hybridizes under stringent conditions to the sequence of (a);
   (c) a portion of the nucleotide sequence of (a) which comprises 100 or more nucleotide residues;
   (d) a portion of the nucleotide sequence of (b) which comprises 100 or more nucleotide residues;
   (e) a DNA sequence which differs from the DNA sequences of (a)-(c) in codon sequence due to the degeneracy of the genetic code; and
   (f) a DNA sequence which differs from the DNA sequences of (a)-(c) due to additions, deletions, or substitutions of nucleic acid residues in said DNA sequence,

   wherein said polypeptide is the functional equivalent of ELF-2.

2. Isolated DNA comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or the complement of SEQ ID NO:1.

3. An RNA sequence corresponding to or complementary to DNA of SEQ ID NO:1 or SEQ ID NO:3.

4. An isolated ELF-2 gene.

5. Isolated DNA which encodes an ELF-2 ligand or a portion thereof.

6. An isolated polypeptide comprising SEQ ID NO:2, SEQ ID NO:5, or the functional equivalent of SEQ ID NO:2 or SEQ ID NO:5.

7. A polypeptide which, when combined with an Eph receptor, mediates signal transduction characteristic of signal transduction mediated by an ELF-2/Eph receptor complex.

8. A probe or primer comprising all or a portion of SEQ ID NO:1 or its complement, or SEQ ID NO:3, or SEQ ID NO:4.

9. DNA comprising a portion of SEQ ID NO:1 or SEQ ID NO:3, wherein the DNA comprises at least eleven consecutive nucleotides which are not a conserved sequence between ELF-2 and ELK-L/LERK-2/CEK5-L.

10. A DNA probe for the detection of ELF-2, the probe comprising DNA which hybridizes to all or a portion of the DNA sequence represented in SEQ ID NO:1 or SEQ ID NO:3, or to all or a portion of a DNA sequence sufficiently homologous to the DNA sequence of SEQ ID NO:1 or SEQ ID NO:3 that it encodes a protein which is the functional equivalent of ELF-2.

11. An ELF-2 fusion protein comprising:
   a) all or a portion of SEQ ID NO:2 or SEQ ID NO:5, or the functional equivalent of either SEQ ID NO:2 or SEQ ID NO:5; and
   b) an amino acid sequence which is not related or homologous to SEQ ID NO:2 or SEQ ID NO:5.

12. An ELF-2 fusion protein comprising:
    a) all or a portion of SEQ ID NO:2 or SEQ ID NO:5, or the functional equivalent of either SEQ ID NO:2 or SEQ ID NO:5; and
    b) an amino acid sequence of a placental alkaline phosphatase.

13. The fusion protein of Claim 12 wherein the alkaline phosphatase is bound to a monoclonal antibody.

14. An antibody or antibody fragment which has specificity for the polypeptide of Claim 1 or its functional equivalent.

15. A polyclonal antibody according to Claim 14.

16. A monoclonal antibody according to Claim 14.

17. A recombinant antibody according to Claim 15.

18. A humanized antibody according to Claim 16.

19. A recombinant expression plasmid comprising:
    a) DNA which encodes an ELF-2 ligand, DNA which is complementary to DNA encoding an ELF-2 ligand, or a portion thereof; and
    b) a promoter,
    wherein the DNA is operatively linked to the promoter which is capable of regulating transcription of an RNA corresponding or complementary in sequence to said DNA.

20. A eukaryotic host cell having incorporated therein the recombinant expression plasmid of Claim 19.

21. A prokaryotic host cell having incorporated therein the recombinant expression plasmid of Claim 19.

22. The recombinant expression plasmid of Claim 20, wherein said plasmid comprises a human gene for the ELF-2 ligand.

23. The recombinant expression plasmid of Claim 20, wherein said plasmid comprises an ELF-2 gene comprising SEQ ID NO:1 or SEQ ID NO:3.

24. A eukaryotic host cell having incorporated therein a recombinant expression plasmid of Claim 19, wherein said plasmid comprises a human gene encoding an ELF-2 ligand.

25. A eukaryotic host cell having incorporated therein a recombinant expression plasmid of Claim 19, wherein said plasmid comprises an ELF-2 gene comprising SEQ ID NO:1 or SEQ ID NO:3.

26. A eukaryotic host cell having incorporated therein an expression plasmid comprising DNA selected from the group consisting of:

(a) DNA comprising SEQ ID NO:1 or SEQ ID NO:3;

(b) a nucleotide sequence which hybridizes under stringent conditions to the sequence of (a);

(c) a portion of the nucleotide sequence of (a) which comprises 14 or more nucleotide residues;

(d) a portion of the nucleotide sequence of (b) which comprises 14 or more nucleotide residues; and

(e) a DNA sequence which differs from the DNA sequences of (a)-(c) in codon sequence due to the degeneracy of the genetic code.

27. A vertebrate model system wherein native ELF-2 expression has been altered by insertion of recombinant DNA or RNA that directly affects ELF-2 transcription or translation.

28. A method of detecting the presence of ELF-2 ligand in a sample of vertebrate cells or tissues, comprising:

a) obtaining a sample of vertebrate cells;

b) treating the sample in a manner that renders RNA encoding the ELF-2 ligand available for hybridization with a complementary DNA or RNA oligonucleotide, thereby producing a treated sample;

c) contacting the treated sample with at least one DNA or RNA probe which is a nucleotide sequence complementary to all or a portion of an ELF-2 gene or mRNA encoded by an ELF-2 gene; and

d) detecting the hybridization of DNA from the sample with the probe,

wherein hybridization is an indication of the presence of ELF-2 ligand in the sample.

29. A method according to Claim 28, further comprising quantifying the ELF-2 ligand in the sample by measuring the extent of hybridization, wherein the extent of hybridization is directly related to the quantity of ELF-2 ligand in the sample.

30. A method according to Claim 28, wherein the probe is associated with a detectable label.

31. A method according to Claim 29, wherein the probe is associated with a detectable label.

32. A diagnostic kit for detecting or quantifying ELF-2 by hybridizing a sample of RNA from an individual with one or more nucleic acid probes, wherein the kit comprises a nucleic acid probe which is a nucleotide sequence complementary to all or a portion of an ELF-2 gene or mRNA encoded by an ELF-2 gene.

33. A diagnostic kit according to Claim 32 wherein the nucleic acid probe is detectably labeled.

34. A method of detecting the presence of ELF-2 ligand in a sample of vertebrate cells or tissues, comprising the steps of:

a) obtaining the sample from an individual;

b) treating the sample in a manner that renders the ELF-2 ligand available for binding to an ELF-2 binding antibody or antibody fragment, thereby producing a treated sample;

c) contacting the treated sample with ELF-2 binding antibody or antibody fragments under conditions appropriate for formation of antibody-antigen complexes; and

d) detecting the presence of antibody-antigen complexes,

wherein the presence of antibody-antigen complexes is an indication of the presence of ELF-2 ligand in the sample.

35. A method of quantifying the amount of ELF-2 ligand in a sample of vertebrate cells or tissues, comprising the steps of:

a) obtaining the sample from an individual;

b) treating the sample in a manner that renders the ELF-2 ligand available for binding to an ELF-2 binding antibody or antibody fragment, thereby producing a treated sample;

c) contacting the treated sample with ELF-2 binding antibody or antibody fragments under conditions appropriate for formation of antibody-antigen complexes; and

d) detecting the amount of antibody-antigen complexes,

wherein the amount of antibody-antigen complexes is an indication of the amount of ELF-2 ligand in the sample.

36. A method of Claim 34 wherein the antibodies are labeled with an enzyme, a radioactive substance, a chromophore, or a fluorochrome.

37. A method of Claim 35 wherein the antibodies are labeled with an enzyme, a radioactive substance, a chromophore, or a fluorochrome.

38. A kit for detecting or quantifying ELF-2, comprising an antibody or antibody fragment which is specific for the ELF-2 polypeptide and a second antibody which binds to the first antibody and is labeled.

39. A method for treating or preventing a disease or condition resulting from the activity of an ELF-2 ligand by administering to an individual in need thereof an ELF-2 binding agent in an amount effective to bind ELF-2 and treat or prevent the disease or condition.

40. A method for treating or preventing a neurological disease or condition by administering to an individual in need thereof an ELF-2 gene in an amount sufficient to treat or prevent the disease or condition.

41. The method of Claim 40 wherein the ELF-2 gene is administered to the individual in such a manner that an ELF-2 ligand is produced in cells of the individual.

42. A pharmaceutical composition for treating or preventing a disease resulting from the activity of an ELF-2 ligand, said composition comprising an agent that binds to the ELF-2 ligand and a pharmaceutically acceptable carrier.

43. A method of inhibiting binding of an ELF-2 extracellular binding domain to an Eph receptor of the ELF-2 extracellular binding domain, comprising contacting the ELF-2 extracellular binding domain with an agent that binds to the ELF-2 extracellular binding domain and prevents further binding of the domain.

44. A method of Claim 43 wherein the ELF-2 extracellular binding domain is contacted with the binding agent in an individual.

45. A method of enhancing ELF-2/Eph receptor activity in a cell or tissue comprising increasing the amount of ELF-2 in the cell or tissue thereby increasing the number of ELF-2/Eph receptor complexes in the cell or tissue.

46. A method of modulating, in a vertebrate animal, the binding of an ELF-2 ligand to its Eph receptor, comprising administering a therapeutically effective amount of the ELF-2 ligand or its functional equivalent, or an inhibitor of the ELF-2 ligand to the vertebrate animal.

47. A method of preventing or treating tumor formation in an individual in need thereof, comprising inhibiting, enhancing, or otherwise modulating the binding of an ELF-2 ligand to its Eph receptor.

48. A method of producing selective neuron development in an individual, comprising increasing or otherwise altering the expression of ELF-2 in a vertebrate cell or tissue.

49. Isolated DNA having a nucleotide sequence corresponding to all or a portion of the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or a homologue of either SEQ ID NO:1 or SEQ ID NO:3, which encodes a protein of vertebrate origin which is strongly expressed in the anterior hindbrain and newly-forming somites of embryos at the early organogenesis stage of development.

50. A method for identifying an Eph receptor of an ELF-2 polypeptide comprising contacting a sample of cells or tissues with the ELF-2 polypeptide, isolating the ELF-2/Eph receptor complex, and isolating and sequencing the Eph receptor.

51. A product for use in therapy comprising any of:

a) an ELF-2 binding agent;

b) an ELF-2 gene;

c) an ELF-2 ligand;
d) a functional equivalent of an ELF-2 ligand; and
e) an inhibitor of an ELF-2 ligand.

52. Use of an ELF-2 binding agent for the manufacture of a medicament for use in binding ELF-2 in the treatment or prevention of a disease or condition resulting from the activity of an ELF-2 ligand.

53. Use of an ELF-2 gene for the manufacture of a medicament for the treatment or prevention of a neurological disease or condition in an individual.

54. Use according to Claim 53, wherein an ELF-2 ligand is produced in cells of the individual.

55. A binding agent that binds to an ELF-2 extracellular binding domain and for use in therapy by inhibiting binding of an ELF-2 extracellular binding domain to an Eph receptor of the domain and preventing further binding of the domain.

56. Use of any of:
a) an ELF-2 ligand;
b) its functional equivalent; and
c) an inhibitor of the ELF-2 ligand for the manufacture of a medicament for modulating the binding of an ELF-2 ligand to its Eph receptor in a vertebrate animal.

57. An agent for use in preventing or treating tumor formation by inhibiting, enhancing or otherwise modulating the binding of an ELF-2 ligand to its Eph receptor.

58. An agent for use in producing therapeutic selective neuron development in an individual by increasing or otherwise altering the expression of ELF-2 in a vertebrate cell or tissue.

```
                                                                    60
TGTCCGCCCCGGAGGATTGGGGGTCGCTGCCCGCGGCCGGTCCCAACGCGTCCCGGAGTG

                                                                   120
CGCAGAACTGGGAGCGGCTTGGGCATGCCATGGCCCGGTCCAGGAGGGACTCTGTGTGGA
                                        MetAlaArgSerArgArgAspSerValTrpL

                                                                   180
AGTACTGTTGGGGACTTTTGATGGTTTTGTGCAGAACTGCGATCTCCAGATCGATAGTTT
ysTyrCysTrpGlyLeuLeuMetValLeuCysArgThrAlaIleSerArgSerIleValL

                                                                   240
TAGAGCCTATCTACTGGAATTCCTCGAACTCCAAATTTCTACCCGGACAAGGCCTGGTAC
euGluProIleTyrTrpAsnSerSerAsnSerLysPheLeuProGlyGlnGlyLeuValL

                                                                   300
TATACCCACAGATAGGAGACAAATTGGATATTATTTGCCCCAAAGTGGACTCTAAAACTG
euTyrProGlnIleGlyAspLysLeuAspIleIleCysProLysValAspSerLysThrV

                                                                   360
TTGGCCAGTATGAATATTATAAAGTTTATATGGTTGATAAAGACCAAGCAGACAGATGCA
alGlyGlnTyrGluTyrTyrLysValTyrMetValAspLysAspGlnAlaAspArgCysT

                                                                   420
CAATTAAGAAGGAGAATACCCCGCTGCTCAACTGTGCCAGACCAGACCAAGATGTGAAAT
hrIleLysLysGluAsnThrProLeuLeuAsnCysAlaArgProAspGlnAspValLysP

                                                                   480
TCACCATCAAGTTTCAAGAATTCAGCCCTAACCTCTGGGGTCTAGAATTTCAGAAGAACA
heThrIleLysPheGlnGluPheSerProAsnLeuTrpGlyLeuGluPheGlnLysAsnL

                                                                   540
AAGATTACTACATTATATCTACATCAAATGGGTCTTTGGAGGGCCTGGATAACCAGGAGG
ysAspTyrTyrIleIleSerThrSerAsnGlySerLeuGluGlyLeuAspAsnGlnGluG

                                                                   600
GAGGGGTGTGCCAGACAAGAGCCATGAAGATCCTCATGAAAGTTGGACAAGATGCAAGTT
lyGlyValCysGlnThrArgAlaMetLysIleLeuMetLysValGlyGlnAspAlaSerS

                                                                   660
CTGCTGGATCAGCCAGGAATCACGGTCCAACAAGACGTCCAGAGCTAGAAGCTGGTACAA
erAlaGlySerAlaArgAsnHisGlyProThrArgArgProGluLeuGluAlaGlyThrA
```

FIGURE 1A

720
ATGGGAGAAGTTCAACAACAAGTCCCTTTGTGAAGCCAAATCCAGGTTCTAGCACCGATG
snGlyArgSerSerThrThrSerProPheValLysProAsnProGlySerSerThrAspG

780
GCAACAGCGCGGGGCATTCCGGGAACAATCTCCTGGGTTCCGAAGTGGCCTTATTCGCAG
lyAsnSerAlaGlyHisSerGlyAsnAsnLeuLeuGlySerGluValAlaLeuPheAlaG

840
GGATCGCATCAGGATGCATCATCTTCATCGTCATCATCATCACTTTGGTGGTGCTGCTGC
lyIleAlaSerGlyCysIleIlePheIleValIleIleIleThrLeuValValLeuLeuL

900
TCAAGTACCGCAGGAGACACCGCAAACACTCTCCACAGCACACGACCACGCTGTCTCTCA
euLysTyrArgArgArgHisArgLysHisSerProGlnHisThrThrThrLeuSerLeuS

960
GCACACTGGCCACGCCCAAGCGAGGTGGCAACAACAATGGCTCGGAGCCCAGTGACGTTA
erThrLeuAlaThrProLysArgGlyGlyAsnAsnAsnGlySerGluProSerAspValI

1020
TCATACCGCTAAGGACTGCAGACAGCGTCTTCTGCCCGCACTACGAGAAGGTCAGCGGGG
leIleProLeuArgThrAlaAspSerValPheCysProHisTyrGluLysValSerGlyA

1080
ACTATGGGCACCCGGTGTACATCGTGCAGGAGATGCCCCCACAGAGTCCTGCCAACATTT
spTyrGlyHisProValTyrIleValGlnGluMetProProGlnSerProAlaAsnIleT

1140
ACTACAAGGTCTGAGGCCTGAGACCTGCGCCTCCCAAGGGAACTCGCACCTTGTTCTTGG
yrTyrLysValEnd

1200
GCACGCAGGGACTGCCTGAGCCTGGCTGCGGGGGCAGGATGCCTCCTGGAAGAGCCTGGA

TCTGGACAGTTTTGTAGTCTGTAGCTTTTCCGACCCTGGG 1240

FIGURE 1B

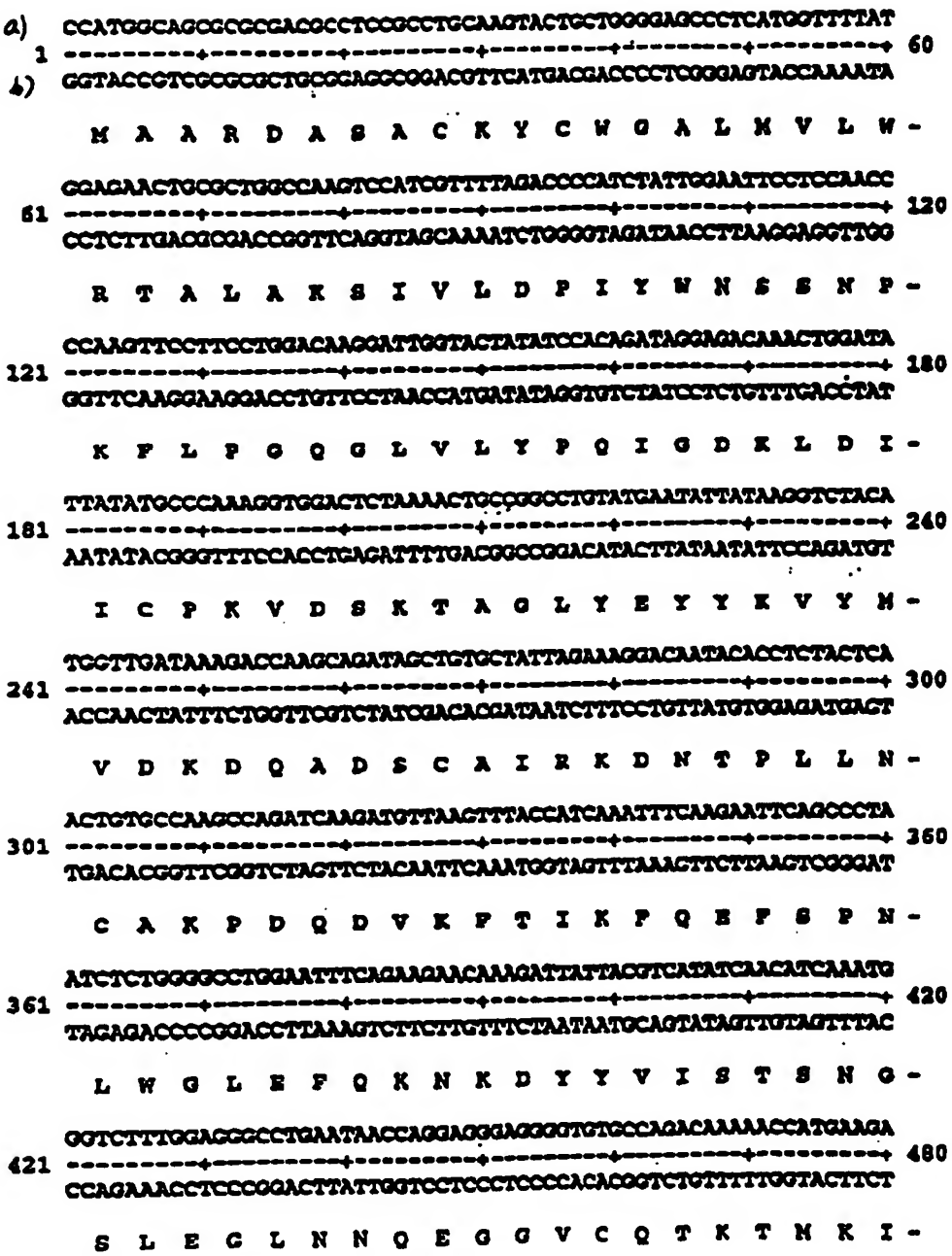
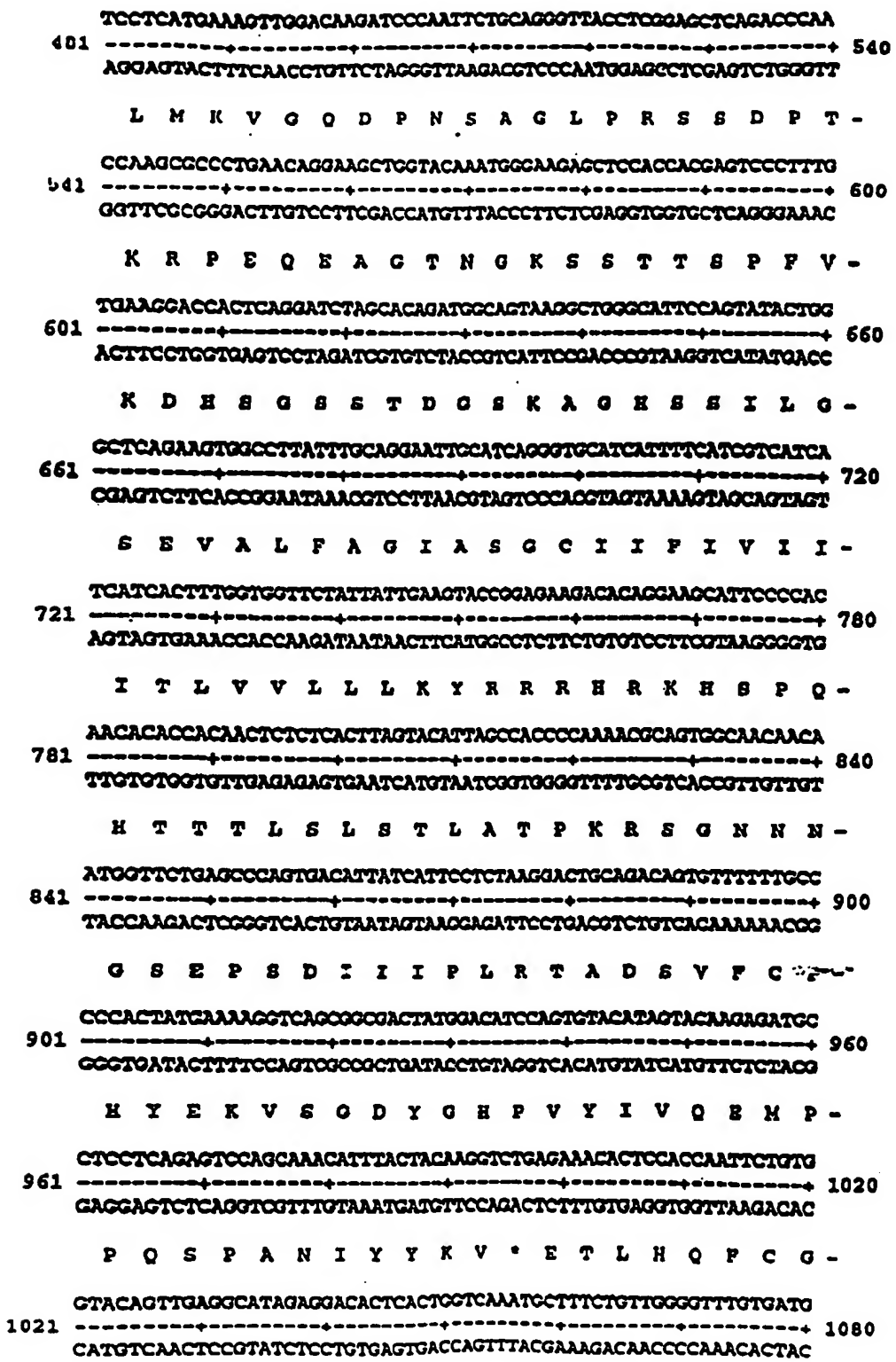

a) CCATGGCAGCGCGCGACGCCTCCGCCTGCAAGTACTGCTGGGGAGCCCTCATGGTTTTAT
1
60
b) GGTACCGTCGCGCGCTGCGGAGGCGGACGTTCATGACGACCCCTCGGGAGTACCAAAATA
M A A R D A S A C K Y C W G A L M V L W -
GGAGAACTGCGCTGGCCAAGTCCATCGTTTTAGACCCCATCTATTGGAATTCCTCCAACC
61
120
CCTCTTGACGCGACCGGTTCAGGTAGCAAAATCTGGGGTAGATAACCTTAAGGAGGTTGG
R T A L A K S I V L D P I Y W N S S N P -
CCAAGTTCCTTCCTGGACAAGGATTGGTACTATATCCACAGATAGGAGACAAACTGGATA
121
180
GGTTCAAGGAAGGACCTGTTCCTAACCATGATATAGGTGTCTATCCTCTGTTTGACCTAT
K F L P G Q G L V L Y P Q I G D K L D I -
TTATATGCCCAAAGGTGGACTCTAAAACTGCCGGGCCTGTATGAATATTATAAGGTCTACA
181
240
AATATACGGGTTTCCACCTGAGATTTTGACGGCCCGACATACTTATAATATTCCAGATGT
I C P K V D S K T A G L Y E Y Y K V Y H -
TGGTTGATAAAGACCAAGCAGATAGCTGTGCTATTAGAAAGGACAATACACCTCTACTCA
241
300
ACCAACTATTTCTGGTTCGTCTATCGACACGATAATCTTTCCTGTTATGTGGAGATGAGT
V D K D Q A D S C A I R K D N T P L L N -
ACTGTGCCAAGCCAGATCAAGATGTTAAGTTTACCATCAAATTTCAAGAATTCAGCCCTA
301
360
TGACACGGTTCGGTCTAGTTCTACAATTCAAATGGTAGTTTAAACTTCTTAAGTCGGGAT
C A K P D Q D V K F T I K F Q E F S P N -
ATCTCTGGGGCCTGGAATTTCAGAAGAACAAAGATTATTACGTCATATCAACATCAAATG
361
420
TAGAGACCCCGGACCTTAAAGTCTTCTTGTTTCTAATAATGCAGTATAGTTGTAGTTTAC
L W G L E F Q K N K D Y Y V I S T S N G -
GGTCTTTGGAGGGCCTGAATAACCAGGAGGGAGGGGTGTGCCAGACAAAAACCATGAAGA
421
480
CCAGAAACCTCCCGGACTTATTGGTCCTCCCTCCCCACACGGTCTGTTTTTGGTACTTCT
S L E G L N N Q E G G V C Q T K T M K I -

FIGURE 2A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401
540
AGGAGTACTTTCAACCTGTTCTAGGGTTAAGACGTCCCAATGGAGCCTCGAGTCTGGGTT
L M K V G Q D P N S A G L P R S S D P T -
CCAAGCGCCCTGAACAGGAAGCTGGTACAAATGGCAAGAGCTCCACCACGAGTCCCTTTG
541
600
GGTTCGCGGGACTTGTCCTTCGACCATGTTTACCCTTCTCGAGGTGGTGCTCAGGGAAAC
K R P E Q E A G T N G K S S T T S P F V -
TGAAGGACCACTCAGGATCTAGCACAGATGGCAGTAAGGCTGGGCATTCCAGTATACTGG
601
660
ACTTCCTGGTGAGTCCTAGATCGTGTCTACCGTCATTCCGACCCGTAAGGTCATATGACC
K D H S G S S T D G S K A G H S S I L G -
GCTCAGAAGTGGCCTTATTGCAGGAATTGCATCAGGGTGCATCATTTTCATGGTCATCA
661
720
CGAGTCTTCACCGGAATAAACGTCCTTAACGTAGTCCCACGTAGTAAAAGTACCAGTAGT
S E V A L F A G I A S G C I I F I V I I -
TCATCACTTTGGTGGTTCTATTATTGAAGTACCGGAGAAGACACAGGAAGCATTCCCCAC
721
780
AGTAGTGAAACCACCAAGATAATAACTTCATGGCCTCTTCTGTGTCCTTCGTAAGGGGTG
I T L V V L L L K Y R R R H R K H S P Q -
AACACACCACAACTCTCTCACTTAGTACATTAGCCACCCCAAAACGCAGTGGCAACAACA
781
840
TTGTGTGGTGTTGAGAGAGTGAATCATGTAATCGGTGGGGTTTTGCGTCACCGTTGTTGT
H T T T L S L S T L A T P K R S G N N N -
ATGGTTCTGAGCCCAGTGACATTATCATTCCTCTAAGGACTGCAGACAGTGTTTTTTGCC
841
900
TACCAAGACTCGGGTCACTGTAATAGTAAGGAGATTCCTGACGTCTGTCACAAAAAACGG
G S E P S D I I I P L R T A D S V F C -
CCCACTATGAAAAGGTCAGCGGCGACTATGGACATCCAGTGTACATAGTACAAGAGATGC
901
960
GGGTGATACTTTTCCAGTCGCCGCTGATACCTGTAGGTCACATGTATCATGTTCTCTACG
H Y E K V S G D Y G H P V Y I V Q E M P -
CTCCTCAGAGTCCAGCAAACATTTACTACAAGGTCTGAGAAACACTCCACCAATTCTGTG
961
1020
GAGGAGTCTCAGGTCGTTTGTAAATGATGTTCCAGACTCTTTGTGAGGTGGTTAAGACAC
P Q S P A N I Y Y K V * E T L H Q F C G -
GTACAGTTGAGGCATAGAGGACACTCACTGGTCAAATGCTTTCTGTTGGGGTTTGTGATG
1021
1080
CATGTCAACTCCGTATCTCCTGTGAGTGACCAGTTTACGAAAGACAACCCCAAACACTAC

FIGURE 2B

```
      T  V  E  A  *  R  T  L  T  G  Q  N  L  S  V  G  V  C  D  D -

   ACGCCTTGTGTGCTAGCATTGACTCAAGCTCAGAGGGAAGAAGGCAACAGCTCTTTCTGT
1081 ---------+---------+---------+---------+---------+---------+ 1140
   TGCGGAACACACGATCGTAACTGAGTTCGAGTCTCCCTTCTTCCGTTGTCGAGAAAGACA

      A  L  C  A  S  I  D  S  S  S  E  G  R  R  Q  Q  L  F  L  * -

   GAGAGCCAACTCGAGCTGGAACAGCTAACCTAGTCTGTAGAATTCCTATCTCAGTGAATC
1141 ---------+---------+---------+---------+---------+---------+ 1200
   CTCTCGGTTGAGCTCGACCTTGTCGATTGGATCAGACATCTTAAGGATAGAGTCACTTAG

      E  P  T  R  A  G  T  A  N  L  V  C  R  I  P  I  S  V  N  R -

   GGTATTCGCTAAAAGCTGGAAGACTTTATGTAGAAGATGCCCATTCTGATTGCTGTACTC
1201 ---------+---------+---------+---------+---------+---------+ 1260
   CCATAAGCGATTTTCGACCTTCTGAAATACATCTTCTACGGGTAAGACTAACGACATGAG

      Y  S  L  K  A  G  R  L  Y  V  E  D  A  H  S  D  C  C  T  L -

   TTGTTACATTCATCATCCTCAAAGCCATGTGCTGCAGGCCTTCAGGCATGTACAAAAGCC
1261 ---------+---------+---------+---------+---------+---------+ 1320
   AACAATGTAAGTAGTAGGAGTTTCGGTACACGACGTCCCGAAGTCCGTACATGTTTTCGG

      V  T  F  I  I  L  K  A  M  C  C  R  P  S  G  M  Y  K  S  Q -

   AGGGGAAGATGGAGTGATCCATGGATTTTAATAGCTCTAGGCATCCTGGGAAGGTGCTGT
1321 ---------+---------+---------+---------+---------+---------+ 1380
   TCCCCTTCTACCTCACTAGGTACCTAAAATTATCGAGATCCGTAGGACCCTTCCACGACA

      G  K  M  E  *  S  M  D  F  N  S  S  R  H  P  G  K  V  L  * -

   AGGATATCAAGCTTGAAATGCAATCTTCTGACTTTTGTGTGTCTCTCTCAGTGCATACTG
1381 ---------+---------+---------+---------+---------+---------+ 1440
   TCCTATAGTTCGAACTTTACGTTAGAAGACTGAAAACACACAGAGAGAGTCACGTATGAC

      D  I  K  L  E  M  Q  S  S  D  F  C  V  S  L  S  V  H  T  G -

   GATTTGTCACACAGACCTTGGTGTCTAAGTAAGACTTGCTCAAGTTCTCTTGCTTTAGTC
1441 ---------+---------+---------+---------+---------+---------+ 1500
   CTAAACAGTGTGTCTGGAACCACAGATTCATTCTGAACGACTTCAAGAGAACGAAATCAG

      F  V  T  Q  T  L  V  S  K  *  D  L  L  K  F  S  C  F  S  P -

   CATCACTGTTCCATTTATTTTTGTTATTCAGGCTCTGCCATCCTTCACATAAGATTTTCT
1501 ---------+---------+---------+---------+---------+---------+ 1560
   GTAGTGACAAGGTAAATAAAAACAATAAGTCCGAGACGGTAGGAAGTGTATTCTAAAAGA

      S  L  F  H  L  P  L  L  F  R  L  C  H  P  S  H  K  I  F  F -

   TTCACTCCACCTCCTGAATCACTAATGGAACATTAATGTTTAGAGATGCGTGCTCCATCC
1561 ---------+---------+---------+---------+---------+---------+ 1620
   AAGTGAGGTGGAGGACTTAGTGATTACCTTGTAATTACAAATCTCTACGCACGAGGTAGG

      H  S  T  S  *  I  T  N  G  T  L  M  F  R  D  A  C  S  I  H -
```

FIGURE 2C

```
     ATCTACTACAGCAGCCTCACTTGAATGGGTAATGCATTTTTAGAAATTGTGTCTGATAGT
1621 ---------+---------+---------+---------+---------+---------+ 1680
     TAGATGATGTCGTCGGAGTGAACTTACCCATTACGTAAAAATCTTTAACACAGACTATCA

       L  L  Q  Q  P  H  L  N  G  *  C  I  F  R  N  C  V  *  *  * -

     AAGGAGCCTTCCAGCCAAAAAGTTAGGCTGTC
1681 ---------+---------+---------+-- 1712
     TTCCTCGGAAGGTCGGTTTTTCAATCCGACAG

       G  A  F  Q  P  K  S  *  A  V  -
```

FIGURE 2D